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PROFOUND NEUTROPENIA ASSOCIATED WITH HEMODIALYSIS

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Thesis presented to

the faculty of

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in partial fulfillment of the requirements

for the degree of

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"The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood...who knows the great enthusiasms, the great devotions; who spends himself in a worthy cause; who at the best knows in the end the triumph of high achievement, and...if he fails, at least fails while daring greatly, so that his place shall never be with those cold and timid souls who know neither victory nor defeat."

J.F. Kennedy

"To live in the world of creations — to get into it and stay in it — to frequent it and haunt it — to think intensely and fruitfully — to woo combinations and inspirations into being by a depth and continuity of attention and meditation — this is the only thing."

Henry James

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To Carol

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I. GENERAL INTRODUCTION

A profound, transient leukopenia has recently been described in patients undergoing hemodialysis (19,28). The white blood cell count usually begins to fall 1-2 minutes after the start of hemodialysis and reaches its lowest value (approximately 25% of pre-dialysis level) after 2-15 minutes of dialysis. By one hour the leukocyte count has returned to normal. The leukopenia is due to the virtually complete removal of neutrophilic granulocytes from the circulating blood. The return to a normal white count is associated with the appearance of large numbers of immature neutrophils in the circulation. Leukocytosis does not follow, nor is there any change in erythrocyte or platelet levels. Patients have not had fever, chills, rash or other symptoms. If dialysis is halted

for five minutes, and then resumed, the leukopenia will recur (18).

This phenomenon was originally observed in uremic patients undergoing chronic hemodialysis with a twin coil dialyzer, a "single-pass" dialyzing apparatus, and a dialyzing fluid of standard composition. It has since been seen in non-uremic patients and in other dialysis units. It occurs with every type of dialyzer investigated, i.e., the Kiil Dialyzer, Travenol "3/4" coil, and Travenol "ultra-flow 145" coil.

The neutropenia is not a result of loss of white blood cells in the dialysis apparatus. If fresh blood is continuously re-circulated around the dialysis circuit *in vitro*, the white count does not fall. And if 1 unit of bank blood is passed through the dialyzing apparatus, collected, and transfused into the patient, a marked leukopenia is seen after just one minute.

This rapidity also suggests that the leukopenia could not be accounted for simply by the removal from the circulation of the leukocytes which have already passed through the dialyzer.

The fall in white count does not seem to be related to the introduction of pyrogen. If sterile lactated Ringer's solution and a sterile dialyzer are used in place of the normal dialysate and dialyzer, the leukopenia still occurs. If the blood compartment of the dialyzer coil is given 5 times the normal rinse with sterile saline (10 liters), the leukopenia still occurs. And if saline alone is passed through the dialyzer and infused into the patient, the white count does not fall.

The leukopenia is not caused by the blood pump or tubing used. If blood is allowed to circulate through the pump and tubing, bypassing the dialyzer,

the patient's white count remains constant. If the dialyzer is then introduced into this circuit, the white count promptly falls.

It is clear, then, that the dialyzer is a necessary factor in the genesis of the neutropenia. However, because dialysis has not been performed without dialysate present, it is not possible to state whether the dialyzer membrane, the dialysate, or both together are necessary for neutropenia to occur.

A second necessary factor is the blood. As stated above, normal saline or lactated Ringer's solution passed through the dialyzer will not cause neutropenia when infused into the patient.

The combination of this "dialyzer factor" plus the "blood factor" produces a "leukopenic factor" which then requires a "patient factor" to produce leukopenia. This is brought out by the observation that the white

count of blood re-circulated around the dialyzer circuit ("blood factor" + "dialyzer factor") does not fall (28).

The purpose of this study was to elucidate further the above factors and their interrelationships. The first part of the work was directed at establishing the dialyzer membrane as the "dialyzer factor". For this purpose and for further experiments, it was necessary to establish an animal model which clearly reproduced this leukopenic phenomenon. The dog was chosen for this.

The second phase of this investigation was undertaken to determine if other plastic surfaces in contact with blood during extracorporeal circulation could induce leukopenia. The cardio-pulmonary bypass apparatus used during open-heart surgery was studied for this purpose.

The third part of the work was an investigation of the role the lungs play in effecting the leukopenia of hemodialysis.

II. GENERAL METHODS AND MATERIALS

A. Dogs

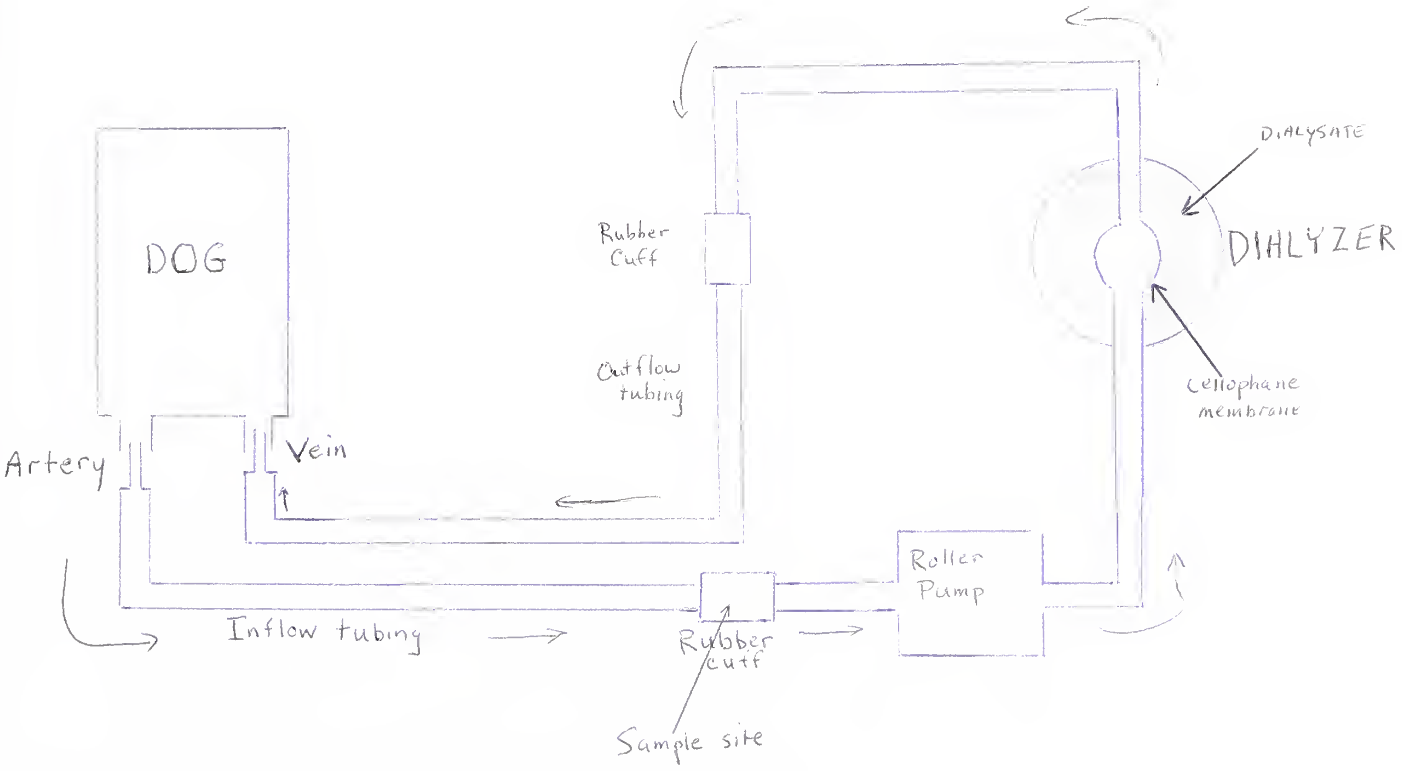
All dogs used in these experiments were healthy mongrels weighing 10-25 kg. They had all been dewormed and quarantined for 21 days. Sodium pentobarbital, 30mg./kg., i.v., was used for induction of anesthesia; 25mg. supplements were given as the dogs became light.

For simple hemodialysis experiments, anticoagulation was maintained with heparin sodium 200 units/kg., i.v., initially, plus 100 units/kg./hour, i.v., thereafter. For dog experiments involving heart-lung bypass the initial heparin dose was 500 units/kg., i.v., with 100 units/kg./hour supplements.

All operative procedures involving the dogs were

carried out under conditions of strict asepsis to avoid introducing pyrogens. The basic cannulation procedure for hemodialysis involved cutdown and isolation of femoral artery and vein, or external jugular vein and carotid artery. The vessels were cannulated with the plastic catheter from an i.v. catheter placement unit.

B. Hemodialysis



Inflow and outflow tubing used was of polyvinyl chloride composition. After cutdown of femoral or neck vessels, the femoral or carotid artery was used for inflow (into the dialyzing apparatus) and the femoral or external jugular vein, respectively, for outflow. Blood was pumped through the circuit by a Sarnes roller pump, usually at a rate of 100cc./minute.

The dialyzer used was a Travenol "ultra flow 145" and dialysate, when indicated, was of standard composition (sodium 132 mEq/L; acetate 35 mEq/L; potassium 2 mEq/L; magnesium 1.5 mEq/L; calcium 2.5 mEq/L; glucose 200 mg%; and chloride 103 mEq/L). The dialyzer and tubing were primed with normal saline.

C. Laboratory Determinations

During simple hemodialysis experiments, blood

samples were obtained by needle puncture of the inflow tubing just distal to the arterial cannulation site. Blood was drawn into heparin-containing vacutainer tubes.

For total leukocyte counts duplicate dilutions were made with a Fisher automatic diluter. Duplicate counts on each dilution were made on a Coulter automatic cell counter. Agreement to within 2.5% was required on the duplicate counts; the separate dilutions also agreed to within 2.5%.

Duplicate hematocrits were done on each sample to provide a basis for correction of hemodilution artifacts in white counts.

Thin smears were made on microscope slides shortly after the samples were drawn; they were later stained with Wright's stain. Differential white counts were performed, counting at least 200 cells on each slide,

unless the white count was very low, and then at least 100 cells were counted.

III. "DIALYZER FACTOR"

Introduction

If blood is passed from the patient through the dialysis tubing, then through the roller pump, and finally returned to the patient, the patient's leukocyte count does not fall; however, if the dialyzer is suddenly interposed between the pump and the patient, a rapid leukopenia follows (28). It is concluded from this observation that there is a "dialyzer factor" necessary for this leukopenia to occur.

The dialyzer consists essentially of a cellophane membrane separating blood from dialysate. This experiment was undertaken to determine if the leukopenic phenomenon would occur in the absence of dialysate. This would then localize the essential "dialyzer factor" to

the dialyzer coil (membrane) itself, rather than any component of the dialysate.

It was felt unsafe to perform human hemodialysis without dialysate. (Glycerol is used in the preparation of the dialyzers, and it must be rinsed out before they are used.) For this reason, and for future experiments, dialysis was performed on a dog. This was found to reproduce well the leukopenia seen with human dialysis.

Methods

Thirteen hemodialyses without dialysate were performed on 7 dogs with the technique described in Part II. However, in all 13 dialyses reported here, the dog's blood was used to prime the tubing and coil. First a 10-20 minute dialysis had been performed with

saline as primer in the tubing and coil. Then 10-20 minutes were allowed for the dog's white count to stabilize, and finally another dialysis was begun, this time with the dog's blood from the previous dialysis filling the coil and tubing. The degree of leukopenia and the changes in differential leukocyte count were identical whether saline or blood was used as primer. However, because it takes about 3 minutes before blood returns to the dog when saline is used as primer, the time of peak leukopenia is delayed. For purposes of comparability, only blood-primed hemodialyses are included here.

As a control, 4 hemodialyses were performed on 2 dogs, with dialysate present. The coils were again primed with blood.

Blood samples were drawn at time 0 (when dog's blood first entered the tubing), 1', 2', 3', 5', 7',

10', and 15'. Total white counts, differential white counts, and hematocrits were performed as described in Part II.

Results

Table 1 shows the leukocyte response to hemodialysis without dialysate in the dog. Absolute white count in cells/mm^3 is shown for the time zero white count, and all further white counts are expressed as a percentage of this initial count. All white counts are corrected for hemodilution using the following formula:

$$\text{WBC}_{\text{corrected}} = \frac{\text{Hematocrit}_{\text{time 0}}}{\text{Hematocrit}_{\text{time x}}} \text{ X } \text{WBC}_{\text{time x}}$$

Hemodilution correction varied from 0 to 10%

There is a precipitous drop in white count during the early minutes of dialysis. The lowest white counts

occurred in the first 3 minutes in 9 of 13 dialyses. The white count dropped to an average 24% (range 12%-43%) of the pre-dialysis level. Table 1 also shows that the white count gradually returns toward normal soon after the peak leukopenia.

Table 2 shows the differential white counts performed on 7 hemodialyses. The peak leukopenia is associated with almost total removal of neutrophils from the circulating blood. The absolute number of circulating mononuclear cells is slightly decreased or unchanged.

The four dialyses performed with dialysate present yielded very similar results. The peak leukopenic white counts were 18%, 20%, 16%, and 28% (mean 20.5%) of pre-dialysis levels. The peak leukopenia occurred at 2', 3', 7.5', and 1' of dialysis. The 7.5' dialysis was performed under conditions of reverse flow, i.e., blood was removed from the dog's vein, circulated

through the dialysis apparatus, and returned to the dog's artery.

Differential white counts were also similar to those of dialyses without dialysate. Again, virtually all of the granulocytes were removed from the circulation with little change in the number of circulating mononuclear cells.

Discussion

The leukocyte response of the dog to hemodialysis is clearly similar to that seen in humans. The time of onset of leukopenia, the degree of leukopenia, the gradual return of the white count toward normal, and the selective removal of granulocytes from the circulation all argue that the mechanism responsible for the dialysis-induced leukopenia in man is the same as that in dog.

The results of this experiment also demonstrate that the leukopenia seen is completely independent of the presence of dialysate. The results seen when dialysate is omitted are similar to those seen when dialysate is present; moreover, they are also identical to those seen in patients when dialysate is present (28).

This then localizes the "dialyzer factor" to the cellulose membrane. It is this which must interact with the blood or some fraction thereof (the "blood factor") to provide the stimulus to leukopenia in the patient.

This can be summarized as:

1) Dialyzer membrane + Blood factor ⟶ Leukopenic factor
2) Leukopenic factor + Patient factor ⟶ Leukopenia

The next study was undertaken to determine if other apparatus commonly used in clinical medicine, with extracorporeal circulation, could produce a "leukopenic

factor". The polyvinyl chloride bubble oxygenator used in open-heart surgery was selected for investigation.

IV. LEUKOCYTE DYNAMICS DURING HEART-LUNG BYPASS

Introduction

When it became clear that the essential "dialyzer factor" was the cellophane dialysis coil itself, a logical question arose: would other plastic materials in clinical use, with extensive surface contact with blood, also lead to a similar leukopenia? The cardiopulmonary bypass apparatus, used during open-heart surgery, was chosen for further investigation. The apparatus consists of a pump-heater and an oxygenator. There are a number of different types of oxygenators, but the one most commonly used at this medical center is a disposable, plastic bubble oxygenator.

The question of early leukocyte response to heart-lung bypass was a virtually uninvestigated one.

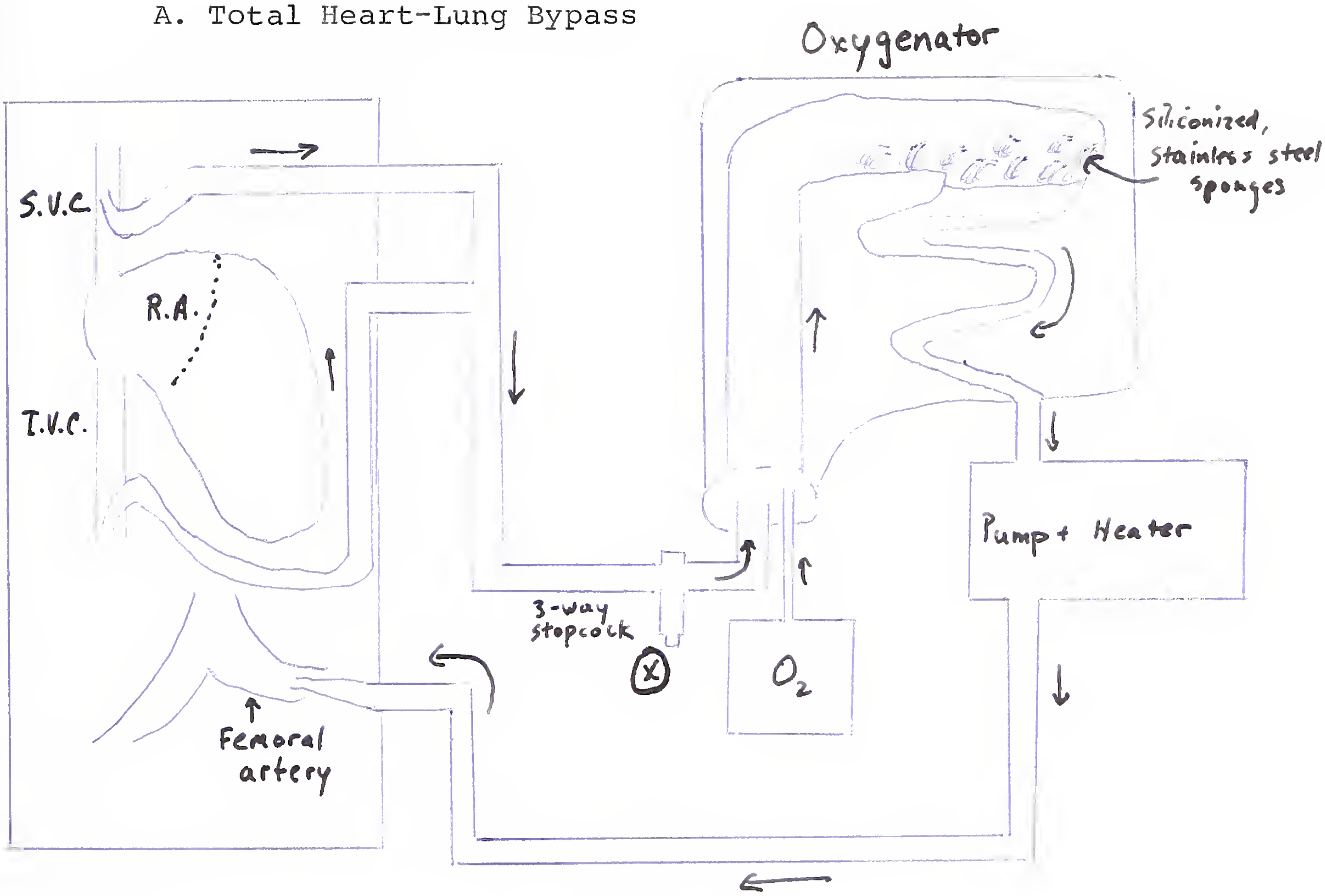
The studies of human and dog bypass experiments all involved late sampling, with the first sample usually obtained at 1 hour, and at the earliest, 15 minutes, after the start of bypass.

This study was in two parts. The first was concerned with delineating the changes in total and differential white counts in humans undergoing total heart-lung bypass.

When it became clear that the leukocyte response was not of the same character or degree as that seen in hemodialysis, one possible explanation was that cardiopulmonary bypass was diverting blood away from the leukopenia-effector organ — the lung. The second part of this work involved shunting part of the cardiac output through the pump-oxygenator while still allowing the rest of the blood to pass through the heart and lungs. This work was carried out in the dog.

Methods

A. Total Heart-Lung Bypass



Ⓧ = Sampling site

As shown in the diagram, both superior and inferior vena cavae are cannulated. Secure ties are placed around the cannulae so that no blood is allowed

to enter the heart.

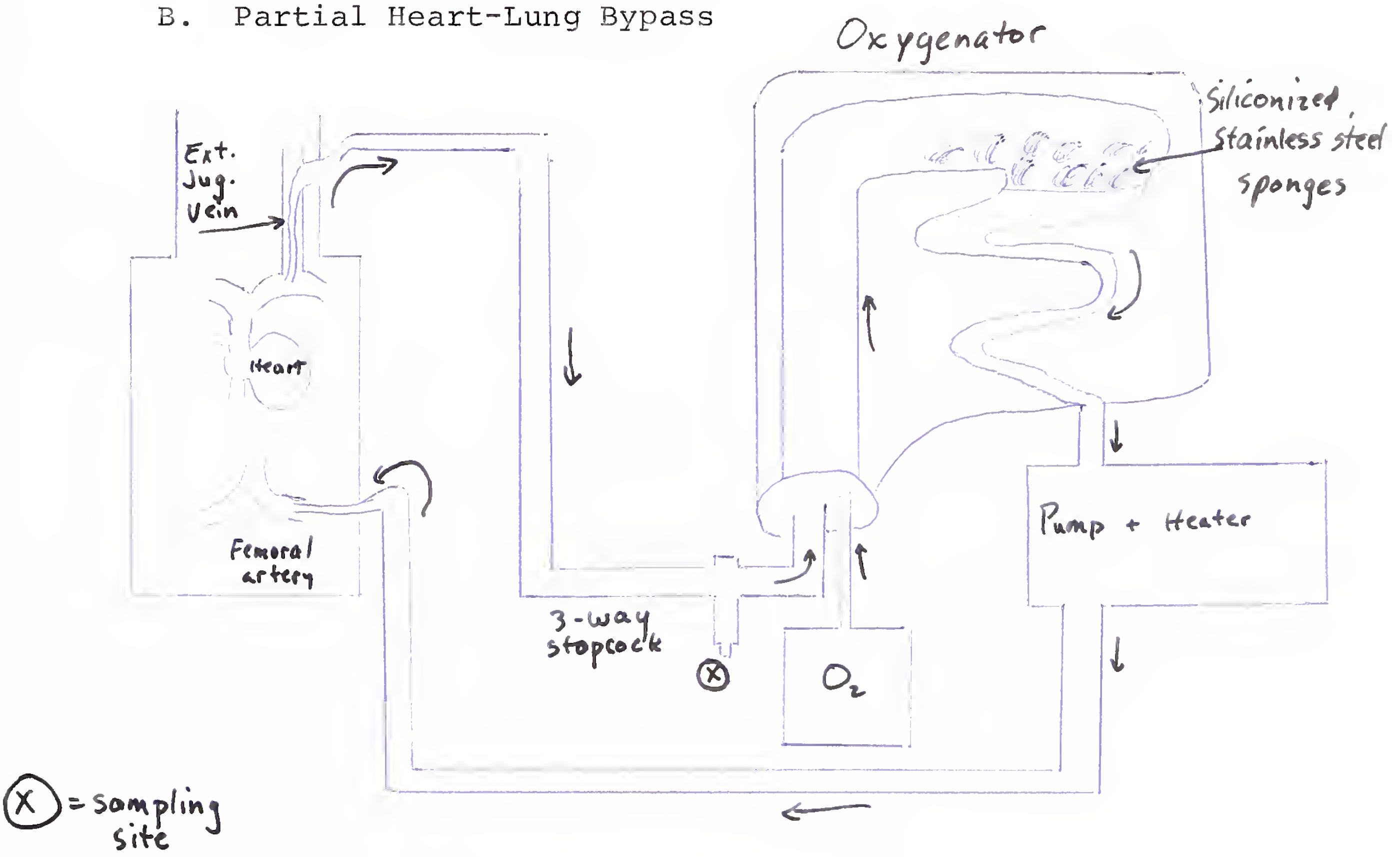
Another cannula, not shown in the diagram, diverts coronary sinus flow into the pump-oxygenator so that no blood enters the lungs via the pulmonary artery. Blood flows by gravity from the cannulae into the oxygenator where O_2 is bubbled through the blood column at 6 liters/minute. The oxygenator is primed with about 1500cc. of D5-0.2N NaCl. The flow rate of blood varies with the size of the patient, but averages about 4 liters/ minute in an adult. After passing through the oxygenator the blood is heated and pumped back to the patient via the femoral artery.

Blood samples were drawn with disposable, plastic syringes from a 3-way stopcock in the line just proximal to the oxygenator, as shown in the diagram. Samples were immediately transferred to heparin-containing vacutainer tubes and total white counts, differential white

counts, and hematocrits were performed as described in Part II.

These studies were performed during 5 randomly selected open-heart operations at Yale-New Haven Hospital. Samples were drawn before bypass began, at the start of bypass (when blood first entered the apparatus), and at 30", 60", 90", 2', 3', 4', 5', 7.5', 10', 15', 30', 45', 60', and 90'.

B. Partial Heart-Lung Bypass



Eight partial heart-lung bypasses were performed on 4 dogs by removing a portion of the venous return to the heart (via jugular vein cannulization), shunting this blood through the pump-oxygenator, and returning the blood to the circulation (via femoral artery cannula). A roller pump was used; it was of the same design as that used for human heart-lung bypass. The bubble oxygenator was of the type and manufacture as that used in human bypass procedures. The blood was not heated. Oxygen was bubbled by tank through the blood column in the oxygenator at a rate of 2-3 liters/minute. The apparatus was primed with 20cc./kg. of normal saline. Blood flow was maintained at 550cc./min. If more than one bypass experiment was performed in the same dog, 15-30 minutes were allowed between experiments so that the white count would stabilize.

Blood samples for hematocrit, total white count,

and differential white count were drawn from a 3-way stopcock in the line leading from the external jugular vein to the bubble oxygenator. 2cc. samples were drawn into disposable, plastic syringes and transferred immediately to heparin-containing vacutainer tubes. Samples were drawn just as blood entered the oxygenator and at 1', 2', 3', 4.5', 6', 10', 15', and 20'. The above-mentioned blood studies were performed using the methods described in Part II.

Results

A. Total Heart-Lung Bypass

Graph 1 shows the response of the total white count to complete heart-lung bypass in the human. White counts are expressed as a percentage of the time 0

(as blood first enters the oxygenator) white count. Hemodilution artifacts have been eliminated by correcting all white counts in a given experiment to a single, arbitrary hematocrit as follows:

$$\frac{\text{Correction Hematocrit}}{\text{Hematocrit}_x} \times \text{WBC}_x = \text{Corrected WBC}_x$$

The hematocrit usually fell to 70%-80% of the pre-bypass level.

The total white count is seen to drop within the first 2 minutes to an average of 62.4% (range 41%-78%) of pre-bypass levels. It gradually returns toward normal during the rest of the operation.

The differential white counts before bypass and at the lowest white count are shown in Table 3. There is clearly no preferential removal of granulocytes or mononuclear cells as the white count falls.

B. Partial Heart-Lung Bypass

Graph 2 and Table 4 depict the changes in total leukocyte count during partial bypass. Again, white counts are expressed as a percentage of time 0 white count; hemodilution artifacts are eliminated as in Part A.

Lowest white counts are seen from 2' to 15' after the start of bypass. The mean fall in white count is to 75.5% (range 52%-86%) of pre-bypass levels. There is not, however, a clear trend toward leukopenia with a progressive fall in white count and a later, gradual return. In 7 of the 8 experiments, white counts greater than pre-bypass white counts occurred in the middle of the experiment, independent of any trend. After Experiment 26 the bypass tubing was disconnected from the dog's cannulae and blood was continuously re-

circulated around the bypass apparatus *in vitro* for 15 minutes. Samples taken from the tubing during this period showed no change in white count.

This *in vitro* re-circulation served the purpose of increasing the surface contact of blood and oxygenator. Thus, immediately after this 15' period was finished, the cannulae were reconnected and Experiment 27 was begun. As can be seen in Table 4, the results of Experiment 27 were little different from the other partial bypasses.

Discussion

Although a drop in white count is seen during the early minutes of total heart-lung bypass, it is different in kind and degree from that seen in hemodialysis. The maximum drop in white count (expressed

as a percent of the initial white count) is significantly less in the bypass group than in the hemodialysis group ($p < 0.05$). Of note also is the fact that the drop in white count seen in heart-lung bypass is caused by the equal disappearance of granulocytic and mononuclear cells, as opposed to the selective neutropenia seen with dialyzing apparatus.

Because it was thought that the absence of the lungs in the total heart-lung bypass circuit might be masking the leukopenic effect, partial heart-lung bypass was performed in the dog. As the dog had been shown in the previous experiment (Part III) to demonstrate well the leukopenia of dialysis, it was expected that if a similar mechanism were operating during bypass, it would become apparent under these conditions.

Graph 2 and Table 4, however, show that the drop in white count with partial bypass was, if anything, less

marked than that seen with total bypass. And Table 5 shows that again there is not a preferential removal of granulocytes from the circulation. This evidence suggests that the mediator of the dialysis-induced leukopenia is *not* activated under conditions of extracorporeal circulation through the bubble-oxygenator.

Alternatively, one could conclude that the mechanism responsible for the leukopenia of dialysis is not an all-or-none mechanism; and in the case of heart-lung bypass, it is not activated to the same degree as in dialysis, perhaps because there is far greater surface contact of blood with dialysis membrane than with the bubble-oxygenator.

Although no one had examined the white count during the early minutes of heart-lung bypass, there have been a number of reports of changes in white counts during the later stages (15 minutes - 24 hours).

Stephenson and Gollan have observed a marked transient leukopenia during total heart-lung bypass in the dog. The former observed a fall to 36% of pre-perfusion levels under conditions of hypothermia, and to 50% under normothermia (38). However, the fall in white count was noted only in the post-perfusion (after at least 30 minutes of bypass) blood sample. No samples during perfusion were reported. He attributed the leukopenia to trapping of white cells in the oxygenator. Gollan observed post-perfusion white count decreases to 30% of pre-perfusion levels and also attributed this to adherence of white cells to the extracorporeal circuitry (20). Although in both studies the pump-oxygenator was primed with a diluting fluid, the fall in hematocrit was not of a magnitude equal to the leukopenia. It is difficult to conclude that this leukopenia, seen at least thirty minutes after the

start of bypass, is similar in genesis to that of dialysis, especially since the higher flow rates through a pump-oxygenator might be expected to accelerate the course of the white cell changes. In addition, Andersen found only a slight *increase* in white count in 9 dogs undergoing total heart-lung bypass (2).

As regards partial heart-lung bypass, Melrose, Galletti, and Brinsfield et al. have observed transient leukopenia in dogs. Melrose observed a fall to 59% of pre-perfusion values, but his earliest measurement was at 30 minutes after the start of bypass (31). Galletti saw an average fall to 34% but made his first measurement at 1 hour (16). And although Brinsfield's first determination was at 15 minutes, he observed the lowest levels at 30 minutes to 1 hour (9).

It is questionable whether the leukopenia observed in the above investigations bears any relation to the

present studies. In both the heart-lung bypasses and hemodialyses reported here, the lowest white counts are seen much earlier and the white count has rebounded well toward normal by the time the above investigators have observed their minimal readings.

There are, then, a number of differences between the leukopenia seen with dialyzing apparatus and that seen with heart-lung bypass. First, the drop in white count is significantly greater during dialysis than during bypass. Second, the leukopenia of dialysis is associated with a selective neutropenia, whereas during bypass the differential white count remains essentially unchanged throughout. Third, during dialysis the white count continues to fall, in a consistent manner, until a low point is reached, after which the leukocyte level gradually returns to normal. During bypass, however,

there is not such a clear pattern; the white count rises and falls in an inconsistent fashion, and it is common to see values greater than controls, occurring sporadically in the middle of a leukopenic trend.

There are a number of dissimilarities between hemodialysis and heart-lung bypass which may help explain the differences in the leukopenias produced. In human cardio-pulmonary bypass, the blood does not circulate through the lungs; Part V will show that the lungs are of major importance in effecting the leukopenia of dialysis. The above partial heart-lung bypass experiments, however, demonstrate that even with the lungs in the circulation, the bypass-induced leukopenia is still different in kind and degree from the dialysis-induced leukopenia.

There are two other essential differences between the partial heart-lung bypass apparatus and the dialysis

apparatus as used in the above experiments. First, in the bypass procedure venous blood is passed through the apparatus and returned to a systemic artery, thereby passing through a systemic capillary bed *before* reaching the lungs. In hemodialysis, on the other hand, arterial blood is passed through the apparatus and returned directly to the lungs via a peripheral vein. This does not appear to be a significant factor for two reasons. Firstly, if dialysis is performed on dogs, using venous blood and returning it via a peripheral *artery*, the changes in white count are similar to those seen in "routine" dialysis[1]. Secondly, similar profound, transient leukopenias have been produced by the intra-arterial injections of agents known to cause a leukopenia mediated by pulmonary sequestration. For both of these reasons it seems that even if the lungs are the major

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[1] In 4 such dialyses the white count fell to 20%, 25%, 40% and 45% of the pre-dialysis values. The lowest white counts were seen by 3-5 minutes of dialysis.

effector-organ, it is not necessary for the "leukopenic factor" to pass through the pulmonary capillary bed before any other. Therefore, the fact that during partial bypass the blood is returned to a systemic artery should not be significant.

Other than the direction of flow, the only other essential difference in the two procedures is that in heart-lung bypass the blood passes through the polyvinyl chloride bubble-oxygenator instead of through the cellulose membrane of the dialyzer. This difference, then, ("dialyzer factor" vs. "oxygenator factor") would seem to account for the dissimilarities seen.

Again, they differ not only in degree, but in kind also. One is a selective neutropenia; the other is non-specific. One shows a continuous, progressive, repeatable fall in white count; the other shows a more inconsistent and erratic leukopenic trend. This evidence

perhaps suggests that the two leukopenias are mediated by different final pathways. This is supported by the observation that a host of other agents (see Part V) can induce acute leukopenia, and the pattern of leukopenia produced is similar to that seen in dialysis.

V. SEQUESTRATION OF LEUKOCYTES IN THE LUNG DURING HEMODIALYSIS

Introduction

The lung acts as the major organ in the removal of leukocytes under a variety of conditions, such as circulation through a Starling heart-lung preparation (1); cardiac catheterization (1); respiratory maneuvers (8); physiological conditions (8); injection of hydrophile colloids (39), nicotinic acid (8), saccharated iron oxide (43), heterospecific blood (8), and bacteria (3); and during systemic anaphylaxis (40). Under some of these conditions the leukopenia is rapid and transient, as is that seen with dialysis. The liver and spleen have been shown to play a lesser role in effecting leukopenia (8).

The purpose of this study, then, was to examine

directly the role of the lungs in effecting the neutropenia of dialysis.

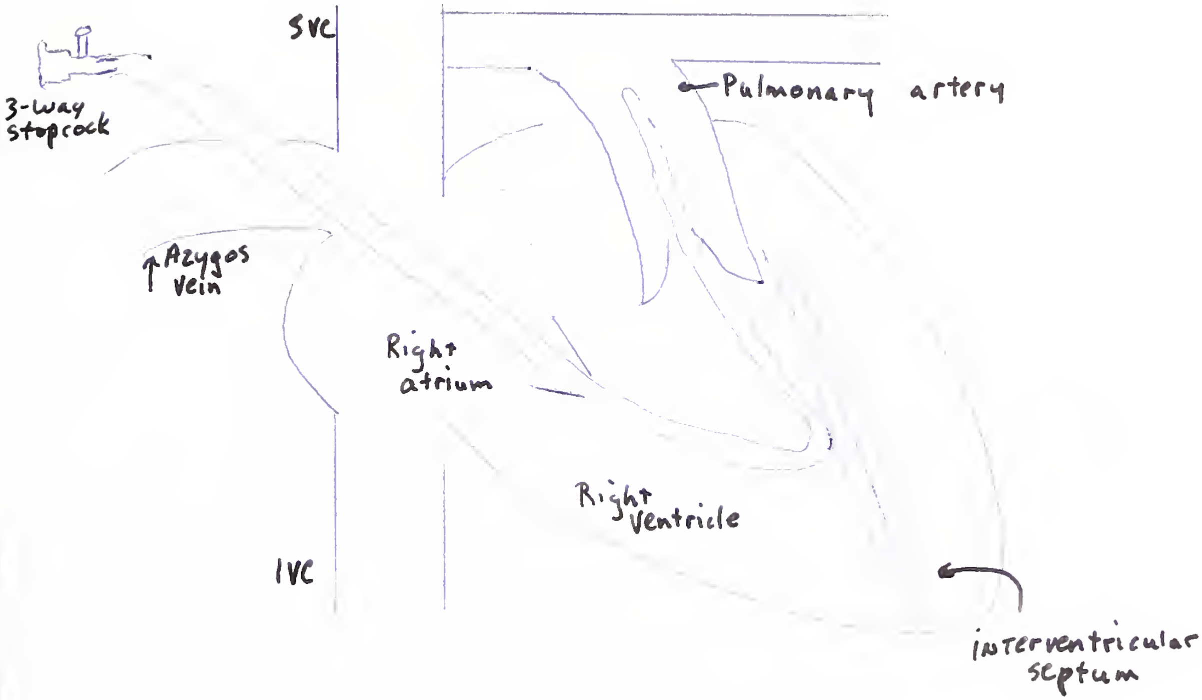
The basic plan was to measure simultaneously the white count of blood entering and blood leaving the lung before, during, and after hemodialysis. If the lungs were indeed responsible, it was hoped that this experiment would demonstrate a dramatic drop in white count across the lungs as the peripheral white count fell during dialysis.

Methods

Six femoral artery to femoral vein hemodialyses were performed in 4 dogs by the usual technique (see Part II). It was assumed that femoral artery blood would be the same as pulmonary blood with respect to total and differential white count. It was also

assumed that transit time between the pulmonary vein and femoral artery was negligible.

The problem of obtaining pulmonary artery blood was more difficult. Because of the unknown, possibly irregular effects of the various organs upon the white count during hemodialysis, it was necessary to obtain samples which were homogeneous, i.e., had passed through a mixing chamber, in this case the right ventricle. To this end, right thoracotomy was performed and the azygos vein was exposed at its junction with the superior vena cava.



A sterile #10 Bardic plastic catheter was advanced through the azygos vein into the superior vena cava, through the right atrium, and at least into the right ventricle, if not the pulmonary artery. The catheter was advanced to a point which had been measured externally on the dog's heart to be sufficient to reach the right or left pulmonary artery. The superior and inferior cavae were carefully palpated to ascertain that the catheter had entered the heart. The right atrium was carefully palpated to be certain that the catheter had not coiled upon itself there. It was then assumed that if the proper length catheter had been advanced, and if the catheter had not been mistakenly detained in the cavae or right atrium, then it most certainly would be in the right ventricle or pulmonary artery. This was confirmed in the two post-mortem examinations performed.

The pulmonary artery samples were taken after first disposing of the 1cc. measured dead space of the Bardic catheter plus the attached sterile, siliconized 3-way stopcock. During dialysis, femoral artery samples were drawn from the rubber cuff sampling site on the inflow tubing (see diagram, Part II, B). However, during control periods the blood column was not moving in the inflow tubing. For sampling purposes, a sterile, siliconized 3-way stopcock was connected to the femoral arterial cannula. During control periods samples were taken after first disposing of the 0.3cc. measured dead space of the arterial cannula plus stopcock.

The first part of each experiment consisted of a 20-40 minute control period with all cannulae in place and the dog's chest open, but with the dialysis pump off. 6-8 samples were drawn simultaneously from pulmonary artery and vein (= femoral artery) during this

control period.

In the first 4 experiments, dialyses were carried on for 15 minutes with simultaneous pulmonary artery and vein samples at time 0 (as blood entered dialysis apparatus), 1', 2', 3', 4', 5', 7.5', 10', and 15'. When 2 experiments were performed on the same dog on the same day, a 10 or 15 minute waiting period between dialyses was allowed. During this period simultaneous pulmonary artery and pulmonary vein samples were drawn at 0, 2.5', 5', 7', and 10' or 0, 2', 5', 8', 11', and 15'.

In the last 2 experiments dialyses were carried on for 30 minutes with simultaneous pulmonary artery and vein samples at 0, (15"), 30", 45", 60", 90", 2', 3', 4.5', 7', 10', 13', (16'), 19', 22', 25', 28', 30'. As soon as dialysis was stopped another series of simultaneous samples was taken at 0 (moment dialysis stopped), 1',

2', 3', 4', 6', 10', 15', 20', and 25'.

Hematocrit, total and differential leukocyte counts were performed as described in Part II.

Results

Results of the total white counts are shown in Graphs 3-6. The bars represent change in white count across the lung, expressed as a percent change, calculated with the formula shown on the graphs. A positive result indicates release of leukocytes from the lung, while a negative result indicates removal by the lung. The continuous line graphs indicate pulmonary artery and vein leukocyte counts.

In 29 control paired samples, the mean change in white count across the lung was 0.17% (range +16% to -12%); the median change was +1.0%.

During each of the 6 dialyses there is a rapid drop in white count, always beginning by 1 minute, at the latest. In each case, as the white count is falling there is a sharp drop across the lungs. The maximum drop across the lungs averages 54.5% (range 48%-67%). The marked drop in white count across the pulmonary vascular bed is consistent within each experiment and from one experiment to the next. Of 40 paired samples taken during the early minutes (as the white count was falling) in these 6 dialyses, there is only one reading that is inconsistent with these findings. This is seen at 7.5' of the first dialysis on Graph 3.

There is no apparent trend toward sequestration or release of leukocytes as the white count returns toward normal, or after dialysis is stopped.

Table 6 illustrates the change in differential white count across the lung at the peak of leukopenia. Three

typical control readings are also included. It is clear that the drop in white count is accounted for by the selective sequestration of granulocytes. During control periods there is essentially no change in differential counts across the lungs.

Discussion

In all 6 dialyses the early fall in white count is attended by a marked sequestration of neutrophils in the lungs. When compared with the change in white count across the lung in control periods, this pulmonary sequestration while the white count falls is statistically significant at the 0.001 level. The timing of the drop across the lungs, the specific sequestration of *neutrophils* in the lungs, and the magnitude of the drop in white count across the lungs, all

suggest strongly that the pulmonary vascular bed plays a major role in effecting the leukopenia. No conclusions can be drawn from the data as to whether this sequestration of leukocytes occurs only in the lungs.

Close inspection of the graphs at the times when the white count is rising just after the peak leukopenia shows that white cells are not being released from the lungs in large numbers. This, plus the increasing numbers of immature granulocytes, suggests that the increase in peripheral white count is due to released leukocytes from extra-pulmonary sites, probably primarily from the marrow. This is in agreement with the findings of Athens et al. (4), Webb (40), and Weisberger et al. (41,42)

Hemodialysis is not unique in inducing an abrupt, transitory leukopenia. Many other agents have been reported to cause this type of leukopenia, and these agents

may be grouped accordingly as:

Pyrogen-associated	References
bacteria	3,10,11,15,22,27,33,34,37
non-specific bacterial products	27
pyrogen	6,44
endotoxin	4
Leukocyte-associated	
leukocytes	41,42
products of disintegrated leukocytes	41
inflammatory exudates	32
"leukopenic factor" isolated from inflammatory exudates	32
leukocytes from an empyema	36
Anaphylaxis-associated	
horse serum	6,14,40
bacteria	3

Colloid-associated	References
fibrinogen	39
nucleic acid	39
starch	39
gum arabic	39
tragacanth	39
salep mucilage	39
dextran	14,30
acacia	14,30
methyl cellulose	24
hepatic glycogen	14
chemically-pure glycogen	26
quartz particles	13
saccarated iron oxide	8,43
gelatin	8,39
plasmoid	8
globulin	39

A discussion of the leukopenias associated with these 4 groups of agents may shed some light on the mechanism responsible for the leukopenia of dialysis.

The first report in the English literature of bacteria-related leukopenia appeared in 1892 (27). Kanthack injected filtered or sterilized cultures of Vibrio metchnikovi, Pseudomonas aeruginosa, and yeasts into the ear vein of a rabbit. He saw a marked (unspecified) leukopenia, associated with fever, and followed by leukocytosis. The leukopenia was seen 1 hour after injection, but this was his first sampling. The sequence of leukopenia and leukocytosis following intravenous or intraperitoneal injection of bacterial products in man and animals has been demonstrated by many workers (3,4,10,11,15,22,27,33,34,37,44). The typical pattern is a drop in white count to 15%-35% of the normal level, usually by the time the first sample has been drawn. This occurs as early as 1 minute after bacterial challenge, but is more common at 1 hour. The leukopenia persists for about 2 hours, followed by a

leukocytosis, reaching a peak at 6 - 12 hours; the white count gradually returns to normal by 24 hours.

Andrewes showed that enteric gram negative bacteria produce the most dramatic leukopenia, with greater than 90% of the neutrophils removed from the circulation at 1 minute (3). The response to killed bacteria is the same as that to living bacteria. Andrewes also showed that the pulmonary capillaries are responsible for most of the sequestration. He sacrificed rabbits 15 minutes after injection of bacteria, and immediately fixed their lungs, liver, spleen, kidneys and marrow in formalin. Compared to control animals there was a great increase in the concentration of leukocytes in the lungs, but essentially no change in the other organs. He also states that "calculation shows that the observed increase in the lung tissue is sufficient to account for the observed decrease in the peripheral circulation." He

suggests that there is something specific about the pulmonary vascular bed, but it must be remembered that the bacteria were introduced into the venous circulation and thus passed through the pulmonary capillaries before any others. Also, the pulmonary capillary bed is far the largest of any organ in the body.

An earlier demonstration of the importance of the lungs in this regard had been provided by Ewing, who retrieved liver, lungs, heart, kidney, spleen, and femur at varying time intervals after ear vein injection (rabbit) of pseudomonas aeruginosa (15). He found that both liver and lungs had a 2-3 - fold increase in leukocyte concentration when the leukopenia was most marked as compared to controls. Bruce also concluded from histological inspection that during peak leukopenia the leukocytes accumulate in the capillaries of the lungs (10).

More recently, Athens et al. have found a mild leukopenia in human volunteers after the intravenous injection of a highly purified, protein-free, lipopolysaccharide endotoxin from Salmonella abortus equi (4). They saw a drop to 67% of control values, but their first sample was taken 1.5 hours after challenge.

As regards the leukopenia of dialysis, it seems untenable to hold that a pyrogen is involved, since the patients have never demonstrated fever or other symptoms. But on the other hand the leukopenias associated with pyrogen and dialysis have much in common. The degree of leukopenia and the time course are similar; in both situations the lungs play the major role in removing the leukocytes; and in both there is a selective neutropenia.

Leukocyte-associated leukopenias comprise the second major group to be discussed. Although this group is presented separately from the pyrogen-associated leukopenias, they may have a similar pathogenesis. Leukocytes themselves have been shown to be a source of endogenous pyrogen (5).

Weisberger et al. obtained a concentrated leukocyte solution by injecting saline into a rabbit's peritoneum and later withdrawing the peritoneal fluid (41). They subjected the white cells obtained to supersonic vibration and centrifugation, and then injected the particle-free supernatant into the same and other rabbits (intravenously). In all cases a prompt neutropenia ensued, beginning 1-5 minutes after injection, reaching a peak leukopenic white count 20-30% of control levels. The leukopenia lasted 30 minutes to 5 hours, and about half the animals then had a leukocytosis beginning 2-4

hours after challenge. Injection of the intact leukocytes produced the same results.

In later studies Weisberger et al. injected similarly obtained P_{32} labeled white cells into rabbits and sacrificed them 30 minutes later (42). He then took radioactive counts of lungs, liver, spleen, kidneys, lymph nodes, thymus, skeletal muscle, femur, skull bones, and brain. The lungs contained by far the greatest part of the radioactivity, both as a fraction of the total radioactivity administered and as a percent of the total activity injected per gram of tissue. Of particular importance was the fact that he obtained the same results when the white cells were injected intravenously, as when they were injected intra-arterially. These findings were also corroborated by histologic examination which showed marked engorgement of lung capillaries with leukocytes. This last study demonstrates that it is not the intravenous

route of administration that the responsible for the predominant role the lungs play in bringing about the leukopenia.

Under the conditions of their experiments whole blood did not produce the leukopenia; moreover, the leukocytes they obtained from the rabbit peritoneum exhibited normal phagocytic activity, normal ameboid activity, and took up vital stains. In search for a mediator of this leukopenia they showed that intravenous histamine could not duplicate their results, nor could intravenous anti-histamines prevent the leukopenia seen with injection of leukocytes.

There are striking similarities between this work and the leukopenia of dialysis. In both situations there is a "blood factor" which appears normal *in vitro*, but which on re-introduction to the host promptly effects a sequestration of neutrophils in the lungs. In addition,

in Weisberger's work, the *in vitro* combination of the white cell solution and whole blood produces no change in the *in vitro* white count. This is again similar to what is seen with dialysis.

Perhaps also related to the active agent in white cells is the leukopenic factor isolated from inflammatory exudates by Menkin (32). The whole exudate, a "leukopenic factor" extracted from the euglobulin fraction of the exudate, and a pyrogen ("pyrexin") frequently found in association with the "leukopenic factor", all caused a drop in white count to about 1/3 of control levels (first sample at 1 hour after intravenous challenge). Careful postmortem examination revealed large numbers of white cells in the alveolar walls of the lungs, the sinusoids of the liver, and the pulp of the spleen.

There is a striking similarity between the above leukocyte-associated leukopenia and that seen with

hemodialysis. The degree of leukopenia is almost identical; the time course, especially as regards the rapidity of the drop in white count, is similar in both; the lungs play a major role in effecting the leukopenia in both; and in both cases there is a selective neutropenia. These similarities strongly suggest a final common pathway.

The third major group of agents causing abrupt leukopenia is related to anaphylaxis. Andrewes first presented suggestive evidence of this in 1910 while investigating the effects upon the white count of injecting bacteria into rabbits (3). He found that bacteria caused a profound leukopenia upon first injection; but if he challenged the same animal with the same strain of bacteria 2-3 weeks after the first challenge, the drop in while count was to 1/10 the previous minimum level. In addition, the rabbits showed

signs of anaphylaxis; indeed, the degree of shock correlated well with the degree of leukopenia.

A more definitive study was done by Webb in 1924 (40). He sensitized dogs with 3 subcutaneous injections of horse serum and challenged them 20 days later with intravenous injection of horse serum. The white count fell to an average of 25% of control levels, beginning as early as 36 seconds after challenge. Differential white counts showed a drop in polymorphonuclear leukocytes from 65% at control to 2% at peak leukopenia. The white count had returned to normal by 2-3 hours. This again is very similar to the pattern seen in hemodialysis.

He checked liver sinusoidal, portal vein, mesenteric vessel, intestinal capillary, splenic capillary and marrow capillary blood white counts and found them to be the same as circulating blood. He obtained

capillary samples by slashing the organs at surgery and doing white counts on the blood dripping from them. When he so examined the lungs he found the lowest levels of leukocytes anywhere; he concluded that the leukocytes were being held tenaciously to the pulmonary vascular bed. He examined the lungs histologically and found leukocytes "unquestionably crowd the pulmonary capillaries in very impressive numbers" during leukopenia. To further corroborate the dynamic importance of this he took simultaneous right and left ventricular blood samples before and during challenge with antigen. Results showed similar white counts during control periods; but after antigen challenge, he demonstrated left ventricular white count slightly less than 50% of right ventricle count as the peripheral white count was falling. By 6 minutes the 2 counts were equal and continued up slowly together. These results are again remarkably similar to

those shown in this present study.

To further substantiate his belief that this reaction was specific to the lungs, he isolated a segment of small intestine, cutting off proximal and distal vascular anastamoses. He cannulated the vein and artery serving this segment, injected antigen into the artery (the rabbit had been sensitized), and measured simultaneous arterial and venous white counts. He found no change in white count across the intestinal segment, concluding that sequestration occurred only in the lungs. However, the mediators of the anaphylactic reaction may not have been present in the intestinal loop. When antigen is injected systemically the active mediators of leukocyte sequestration may be released into the general circulation and the sequestration may be a generalized process.

Essex presented evidence to suggest this. He

implanted a transparent chamber in a rabbit's ear in order to observe microscopically the dynamic situation in the vasculature under various conditions (14). He sensitized a rabbit with horse serum, and on challenge saw greatly increased stickiness and adhesiveness of leukocytes, which adhered to the endothelium and to each other, sometimes blocking the vessel.

Thus the evidence is that quantitatively the lungs remove the bulk of the leukocytes from the circulation during anaphylaxis-induced leukopenia. It is probably, however, a generalized reaction.

In comparing these results with what is seen with dialysis there is again a striking similarity in the time course,the degree of leukopenia, the importance of the lungs, and the specific disappearance of nuetrophils. As in the cases of pyrogen-associated and bacteria-associated leukopenias, these similarities

strongly suggest a final common pathway. Anaphylaxis is also a complex, incompletely understood phenomenon, and the absence of signs of anaphylaxis in dialysis patients does not mean that dialysis and anaphylaxis do not share a final pathway or for that matter, an even more basic mediator.

The fourth group to be discussed is the group of hydrophile colloids. They are all highly viscous, and reduce the suspension stability of the blood (increase the erythrocyte sedimentation rate).

The findings of White, Bierman, Kelly, and Byron, Jr. are typical (43). They injected a colloidal suspension of saccharated iron oxide intravenously into 13 patients. Simultaneous samples from a peripheral artery and either the inferior vena cava, right ventricle, or pulmonary conus showed onset of leukopenia 30 seconds to 5 minutes after injection with the nadir

at 3-20 minutes. The leukopenia was manifest first on the arterial side of the lungs (oxygenated blood), followed in 30-90 seconds by the venous side. The white count usually fell to 15%-35% of control levels, and when differentials were done, the fall in white count was seen to be mostly from a drop in number of circulating granulocytes. A leukocytosis usually followed but did not reach a peak often until 24-48 hours, requiring a week to return to normal.

Essex, using the transparent chamber in the rabbit ear (14), and Veglens, studying guinea-pig, rabbit, and cat mesentary under a microscope (39), both found that with injection of a variety of colloids there follows a tenacious adherence to endothelium by leukocytes ("margination"). They both conclude that this is a generalized response and not peculiar to the pulmonary vascular bed.

Of particular interest is the observation by Hueper that methyl cellulose causes the characteristic leukopenia (24). This is simply the methyl ester of cellulose, the membrane ("dialyzer factor") of the dialysis coil.

Hueper (24,25,26), Essex (14), Bierman (8), and Veglens (39) all concluded that it is the colloidal nature of these agents which is responsible for the leukopenia seen. Hueper simply states that the abnormal colloidal status of the blood and especially the increased viscosity of the blood and particularly the plasma are responsible (24). Veglens showed that certain agents which have some characteristics similar to the colloids, but which do not influence the suspension stability of the blood and which do not have a high viscosity, such as egg albumin, dextrose, and saccharose, produce no change in white count (39).

As in the two previous major groups there is an impressive similarity between the time course and nature of this leukopenia and that seen with dialysis. In addition, the finding that an ester of the cellulose membrane (the "dialyzer factor") itself can reproduce the entire leukopenic phenomenon when introduced into the circulation strongly suggests a similar etiology. The observation that saline passed through the dialysis coil will not cause leukopenia might be explained by the necessity of some agent in the blood to draw the cellulose into solution or suspension.

There is, then, no clear evidence to implicate specifically the pyrogen-associated, the anaphylaxis-associated, or the colloid-associated mechanisms as responsible for the leukopenia of dialysis. On the other hand, the characteristics of all the leukopenias seen under the variety of conditions discussed above

are remarkably consistent:

1) The white count falls to 20%-30% of control values.

2) The fall in white count occurs by 30 seconds to 5 minutes at the latest, if early samples are taken.

3) The fall in count is due to specific removal of neutrophils from the circulation.

4) The lungs always play the major role in the sequestration of the leukocytes.

The stiking similarity of these leukopenias, induced under such a variety of circumstances, strongly suggests that all of them are mediated by a final common pathway, perhaps involving increased adhesiveness of leukocytes (neutrophilic granulocytes in particular) to endothelium and to one another. The bulk of the evidence also shows that although the lungs are quantitatively paramount in effecting the leukopenia,

both in dialysis and the other models, the sequestration of leukocytes under these stimuli is a systemic reaction.

VI. SUMMARY AND CONCLUSIONS

1. Details are presented of a profound, transient leukopenia seen in humans undergoing hemodialysis.

2. 13 dialyses were performed on 7 dogs with similar techniques to those used in humans except that dialysate was not used. It was shown that the dog is an excellent animal model for the dialysis-induced leukopenia. It was also shown that the dialysate is not necessary to produce the leukopenia. This further localized the "dialyzer factor" to the membrane.

3. Total and differential white counts were performed during 5 open-heart operations utilizing a polyvinyl chloride bubble-oxygenator for cardio-pulmonary bypass. A drop in white count was seen

during the early minutes of bypass, but this drop was significantly less than that seen with dialysis. In addition, there was no significant change in differential white count during bypass. The same results were seen when partial bypass was performed on dogs, allowing a part of the venous return to the heart to pass through the bubble-oxygenator, while still allowing blood to circulate through the lungs. It is concluded that the leukopenia occurring during heart-lung bypass may well be mediated by a mechanism different from any discussed in this paper.

4. Simultaneous pulmonary artery and pulmonary vein (= systemic artery) samples were obtained during 6 dialyses on 4 dogs. It was clearly demonstrated that while the white count is falling there is a sequestration of white cells in the lungs. Various other leukopenias are described, and the relation-

ship of these to the leukopenia of dialysis are discussed. Evidence presented suggests that in all leukopenias discussed, there is a shared final common pathway.

APPENDIX

Table 1

Leukocyte Response To Hemodialysis In The Dog

	Exp. No. → Time ↓	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13
	Time 0 WBC/mm^3	6800	9100	8200	9800	13700	7800	5800	8500	14800	8900	10300	8500	5900
Minutes of Dialysis	0	100	100	100	100	100	100	100	100	100	100	100	100	100
	1'	85	▨ 22	26	21	▨ 19	72	▨ 43	71	▨ 15	54	129	75	38
	2'									18	24	37	23	31
	3'	▨ 33	34	▨ 23	▨ 16	24	27	50	34	26	13	47	▨ 16	▨ 30
	5'	57	60	32	29	29	29	56	▨ 33	34	▨ 4':12 5':13	▨ 4':26 5':36	21	35
	7'	60	55	27	46	59	▨ 20	57	41	52	12	49	31	59
	10'	92	56	42	56	62	26	69	58	51	18	57	38	66
	15'				66	70				61	22		50	

Leukocytes, expressed as percentage of pre-dialysis white count

▨ shaded values indicate peak of leukopenia

Table 2

Differential White Counts Before Dialysis And At The Peak Of Leukopenia

	PRE-DIALYSIS				PEAK LEUKOPENIA			
	Differentials			Absolute counts; cells/$_{mm}3$	Differentials			Absolute counts; cells/$_{mm}3$
Exp. No.	S	N	M	$\frac{S+N}{M}$	S	N	M	$\frac{S+N}{M}$
1	11	56	30	$\frac{4550}{2040}$	5	4	86	$\frac{224}{1930}$
2	16	54	26	$\frac{6370}{2360}$	4	6	88	$\frac{200}{1760}$
9	44	41	12	$\frac{12600}{1780}$	15	26	59	$\frac{910}{1330}$
10	42	38	20	$\frac{7130}{1780}$	0	4	96	$\frac{43}{1025}$
11	30	49	21	$\frac{8040}{2160}$	22	26	52	$\frac{1270}{1390}$
12	49	29	21	$\frac{6630}{1790}$	2	8	90	$\frac{136}{1210}$
13	21	44	29	$\frac{3840}{1710}$	6	6	82	$\frac{147}{1450}$

S = segmented neutrophilic granulocytes
N = non-segmented neutrophilic granulocytes
M = mononuclear cells = lymphocytes + monocytes

Table 3

Differential White Counts Before Total Heart-Lung Bypass And At The Peak Of Leukopenia

	PRE-BYPASS				PEAK LEUKOPENIA			
	Differentials			Absolute counts; cells/mm^3	Differentials			Absolute counts; cells/mm^3
Exp. No.	S	N	M	$\frac{S+N}{M}$	S	N	M	$\frac{S+N}{M}$
18	52	24	22	$\frac{15350}{4450}$	45	28	26	$\frac{6940}{2465}$
19	69	17	12	$\frac{16200}{2260}$	61	24	15	$\frac{10800}{1900}$
20					37	42	20	$\frac{8220}{2080}$
21	40	33	26	$\frac{6720}{2390}$	41	46	13	$\frac{3220}{482}$
23	41	45	14	$\frac{13500}{2200}$	38	40	22	$\frac{9450}{2660}$

S= segmented neutrophilic granulocytes
N= non-segmented neutrophilic granulocytes
M= mononuclear cells = lymphocytes + monocytes

Table 4

Leukocytes Responses
To Partial Heart-Lung Bypass In The Dog

	Exp. No.→ Time ↓	#25	#26	#27	#28	#29	#30	#31	#32
	Time 0 WBC/mm^3	17100	16000	16600	11600	9000	3900	8600	6800
Minutes of Bypass	0	100	100	100	100	100	100	100	100
	1'	106	89	86	96	87	98	94	72
	2'	100	86	83	93	81	98	103	87
	3'	86	88	92	107	106	108	83	94
	4½'	85	96	94	87	88	69	81	97
	6'	107	95	92	92	81	75	78	102
	10'	92	107	100	83	86	94	75	83
	15'		97	110	73	124	126	83	52
	20'								63
	25'		106						56
	30'								77

Leucocytes, expressed as percentage of pre-bypass white count.

▨ shaded values indicate peak of leukopenia

Table 5

Differential White Counts Before Partial Heart-Lung Bypass And At The Peak Of Leukopenia

	PRE-BYPASS				PEAK LEUKOPENIA			
	Differentials			Absolute counts; cells/mm^3	Differentials			Absolute counts; cells/mm^3
Exp. No.	S	N	M	$\frac{S+N}{M}$	S	N	M	$\frac{S+N}{M}$
25	33	35	34	$\frac{11600}{5810}$	55	23	21	$\frac{10700}{2840}$
26	46	36	18	$\frac{13000}{2880}$	41	39	19	$\frac{11800}{2800}$
27	36	51	13	$\frac{14400}{2160}$	17	62	21	$\frac{10300}{2750}$
31	53	19	19	$\frac{4900}{1280}$	46	18	30	$\frac{2300}{1070}$

S = segmented neutrophilic granulocytes
N = non-segmented neutrophilic granulocytes
M = mononuclear cells = lymphocytes + monocytes

Table 6

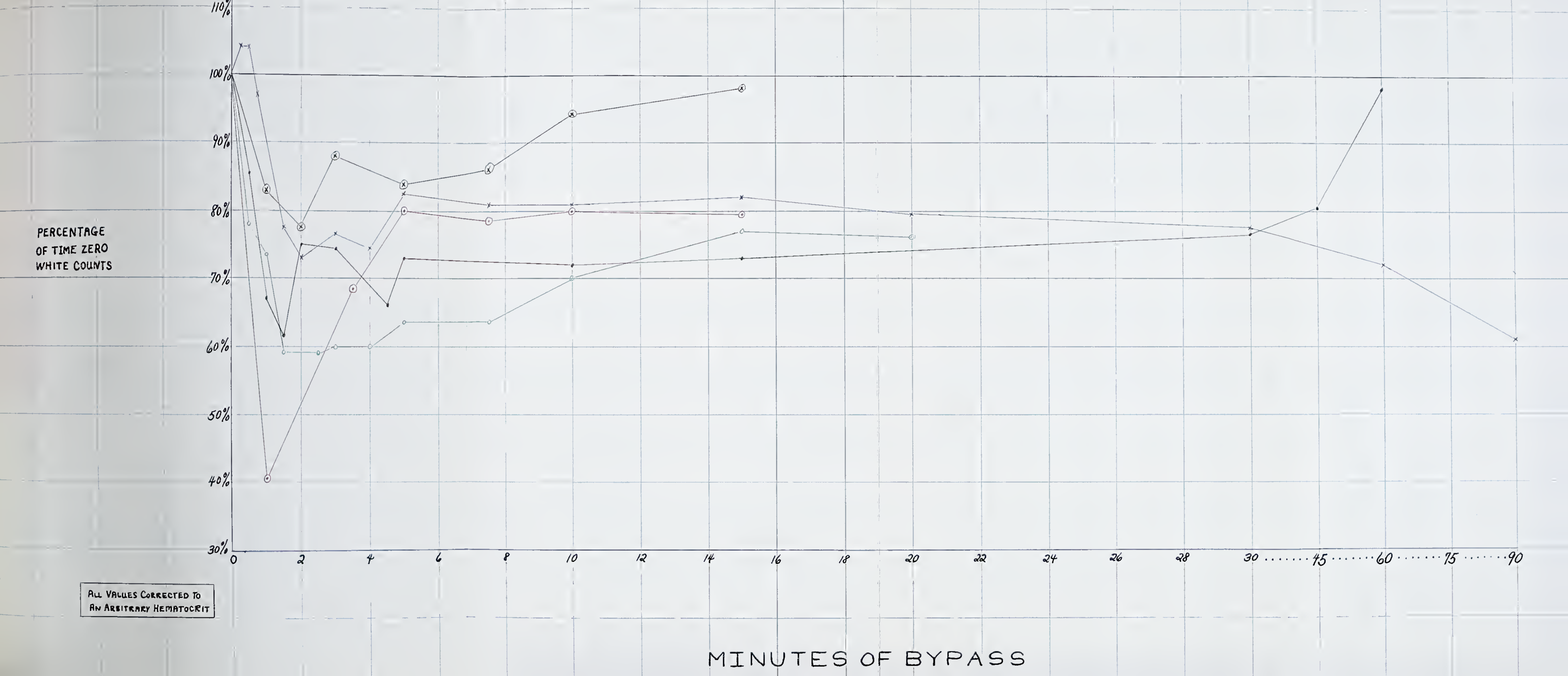
Differential White Counts From Simultaneous Pulmonary Artery And Pulmonary Vein Blood At The Peak Of Leukopenia And During Control Period

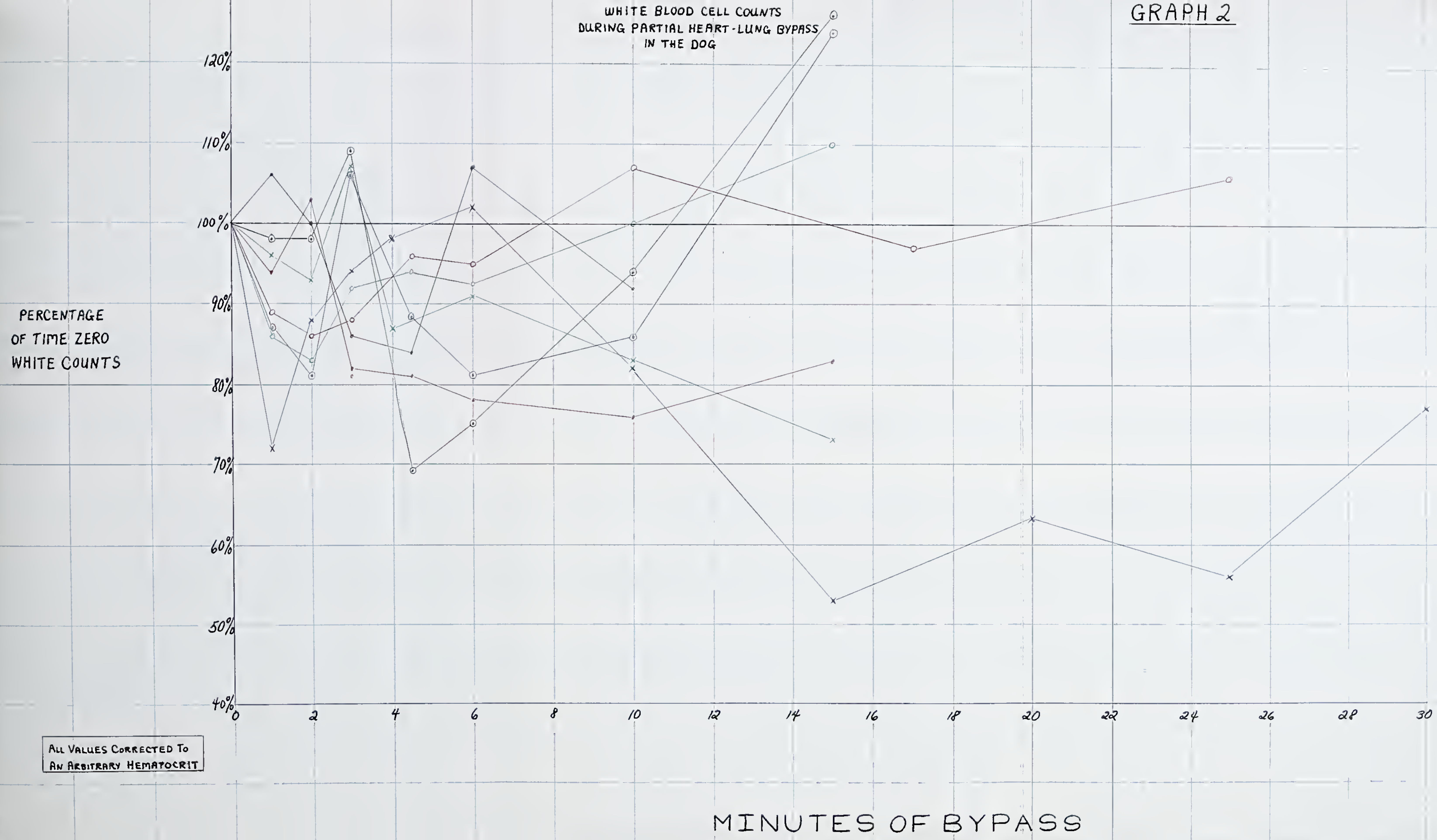
		PULMONARY ARTERY				PULMONARY VEIN			
		Differentials			Absolute counts; cells/mm^3	Differentials			Absolute counts; cells/mm^3
	Exp. No.	S	N	M	$\frac{S+N}{M}$	S	N	M	$\frac{S+N}{M}$
PEAK LEUKOPENIA	34	17	47	30	$\frac{3690}{1260}$	15	26	58	$\frac{900}{1270}$
	35	47	27	25	$\frac{1700}{576}$	0	4	96	$\frac{44}{1060}$
	36	32	47	20	$\frac{4100}{1040}$	22	26	52	$\frac{1345}{1460}$
	37	23	20	53	$\frac{1460}{1800}$	2	8	90	$\frac{150}{1350}$
	38	28	23	43	$\frac{1580}{1330}$	6	6	82	$\frac{240}{1390}$
CONTROL	Control Exp. 35	38	49	13	$\frac{7140}{1065}$	42	38	20	$\frac{7120}{1780}$
	Control Exp. 37	48	32	19	$\frac{9050}{2145}$	49	29	21	$\frac{6650}{1780}$
	Control Exp. 38	34	31	26	$\frac{3770}{1510}$	21	44	29	$\frac{3830}{1710}$

S = segmented neutrophilic granulocytes
N = non-segmented neutrophilic granulocytes
M = mononuclear cells = lymphocytes + monocytes

GRAPH 1

WHITE BLOOD CELL COUNTS DURING TOTAL HEART-LUNG BYPASS IN THE HUMAN

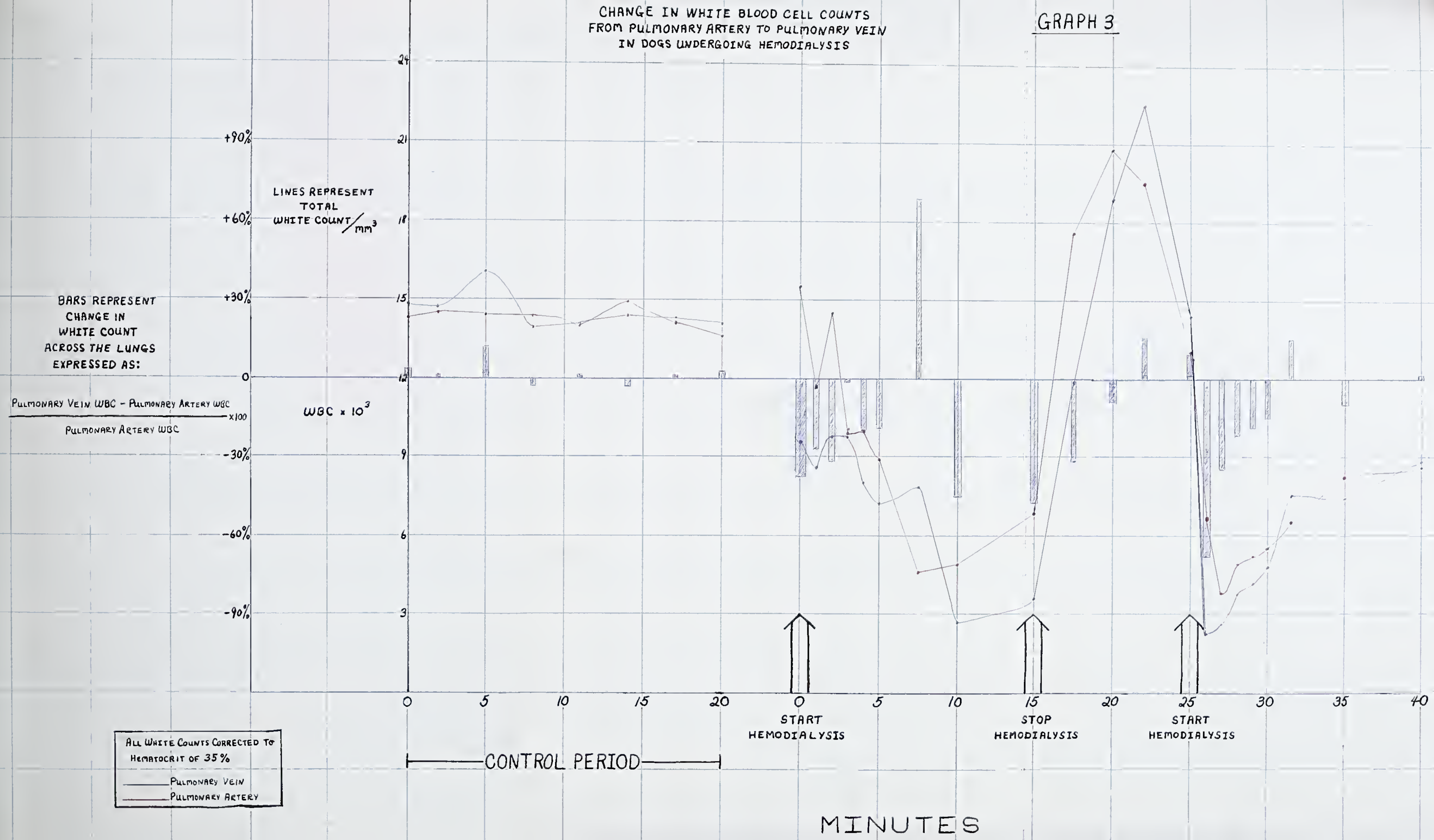




GRAPH 2

WHITE BLOOD CELL COUNTS
DURING PARTIAL HEART-LUNG BYPASS
IN THE DOG

All Values Corrected To
An Arbitrary Hematocrit

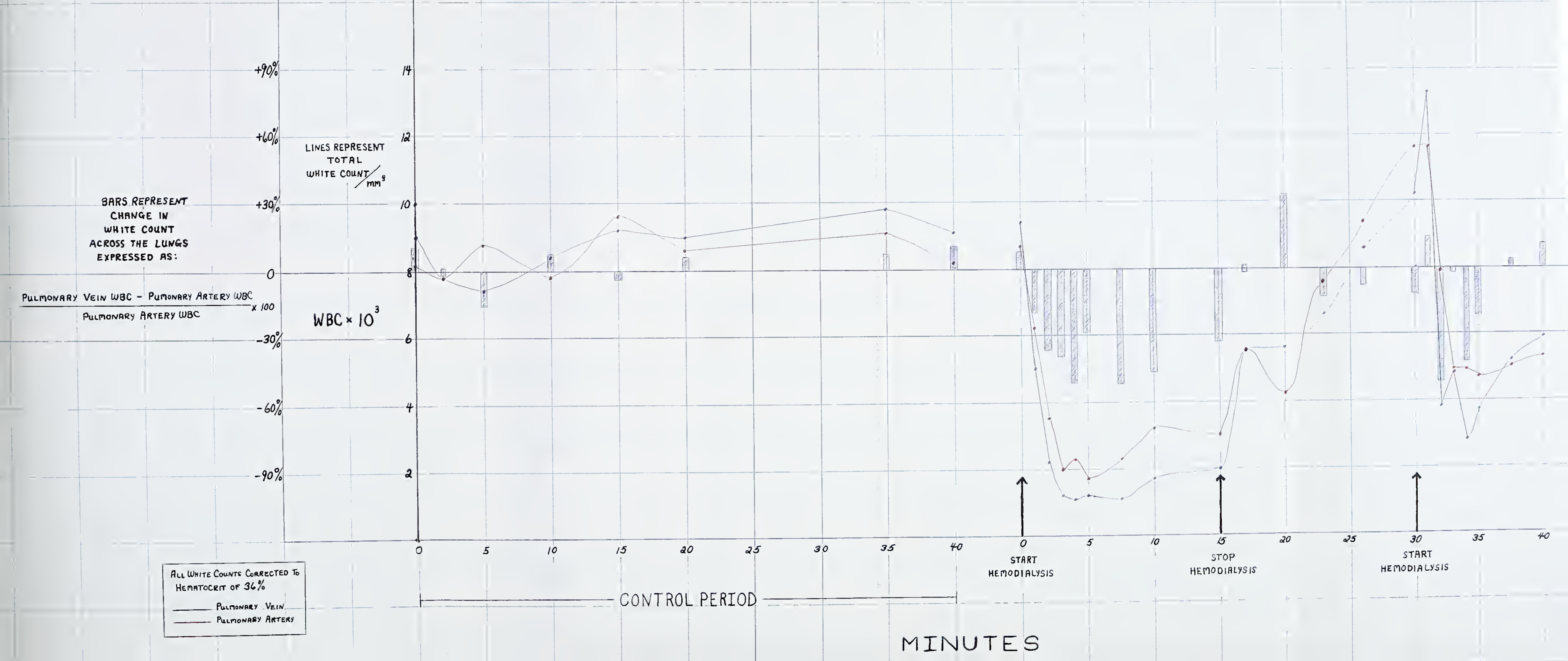


GRAPH 3

CHANGE IN WHITE BLOOD CELL COUNTS FROM PULMONARY ARTERY TO PULMONARY VEIN IN DOGS UNDERGOING HEMODIALYSIS

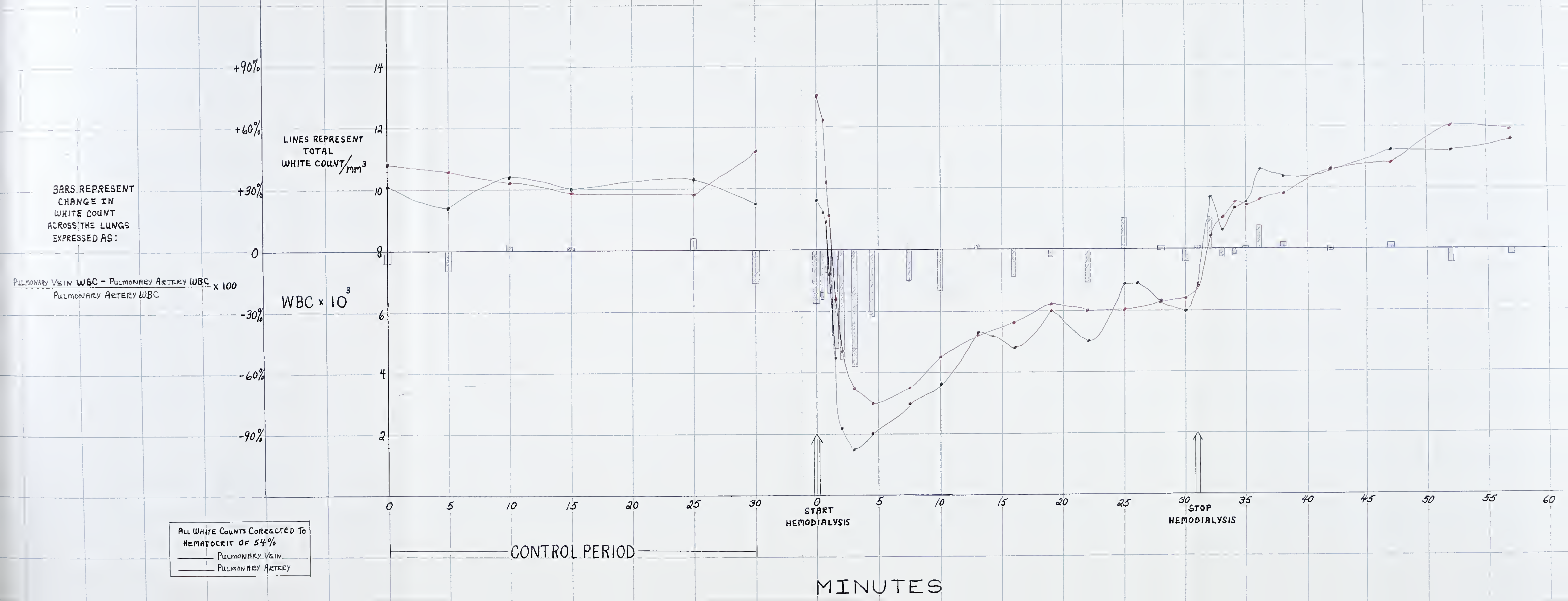
GRAPH 4

CHANGE IN WHITE BLOODCELL COUNTS
FROM PULMONARY ARTERY TO PULMONARY VEIN
IN DOGS UNDERGOING HEMODIALYSIS



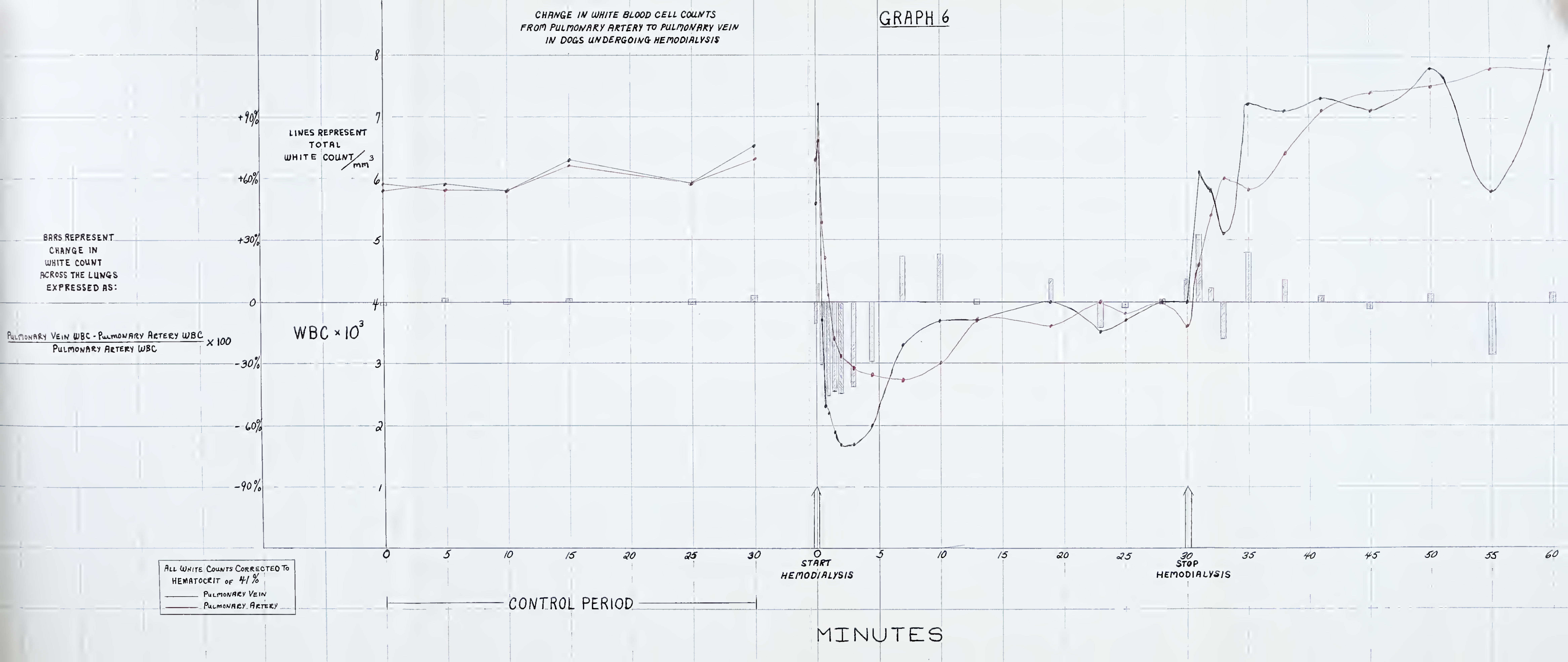
GRAPH 5

CHANGE IN WHITE BLOOD CELL COUNTS FROM PULMONARY ARTERY TO PULMONARY VEIN IN DOGS UNDERGOING HEMODIALYSIS



GRAPH 6

CHANGE IN WHITE BLOOD CELL COUNTS FROM PULMONARY ARTERY TO PULMONARY VEIN IN DOGS UNDERGOING HEMODIALYSIS



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